Salamander Skin Toxins, With Special Reference To *Necturus lewisi*

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ABSTRACT.— Crude aqueous extracts of skin from two specimens of *Necturus lewisi* were found to cause strong symptoms of toxinosis in bioassay mice when injected intraperitoneally. The symptoms resembled those caused by injections of pseudotritontoxin and skin extracts from a variety of other salamanders, but only one bioassay mouse died. In comparison to the effects of injected skin extracts of other salamanders on bioassay mice, the skin of *N. lewisi* is moderately active in causing distress but relatively less lethal. Effects of per os administration on potential natural predators remain to be tested.

INTRODUCTION

The skins of most amphibians—lacking hair, feathers, epidermal scales, dermal armor, or significant keratinization—are relatively thin, heavily glandular, covered with a mucoid coat, and serve as the major physiological interface through which ions, respiratory gases, and water are exchanged with the environment (Whitear 1977). This sort of moist integument in an environment rich in bacteria, fungi, and yeasts is particularly susceptible to invasion by microorganisms and to major physiological disruption if the protective mucoid coat is damaged. In addition to containing many physiologically active regulatory chemicals, the skin is bathed in mucus and other glandular products that may contain a variety of chemical materials—proteins, peptides, steroids, alkaloids, and simple biogenic amines (Cei et al. 1967, 1968; Daly et al. 1977; Daly and Heatwole 1966; Erspamer et al. 1962, 1964; Habermehl 1974; Jaussi and Kunz 1978; Flier et al. 1980; Mensah-Dwumah and Daly 1978; Endean et al. 1975). Some of these chemicals have an antimicrobial function (Habermehl 1975; Habermehl and Preusser 1969, 1970; Preusser et al. 1975). Others may function as stress-warning markers (Hedberg 1981). In many species the chemicals have adaptive significance in rendering the animals noxious or toxic to predators, or provide tastes and smells that predators associate with noxious or toxic effects (Brandon

and Huheey 1981; Brandon et al. 1979a,b; Brodie 1968a,b, 1971, 1977; Brodie and Gibson 1969; Brodie and Howard 1973; Brodie et al. 1974, 1979; Dodd et al. 1974; Dodd and Brodie 1976; Hensel and Brodie 1976; Howard and Brodie 1973; Huheey 1960; Hurlbert 1970; Nickerson and Mays 1973; Pough 1971; and Webster 1960).

Among salamanders, skin toxicity has been examined in detail in Asiatic, European, and North American salamandrids and to a lesser degree among plethodontids. Wakeley et al. (1966) found tetrodotoxin in significant amounts in two species of *Cynops*, three species of *Taricha*, and one species of *Notophthalmus*; trace amounts were found in four species of *Triturus*. Brodie et al. (1974) found a species of *Paramesotriton* also to have highly toxic skin, apparently because of tetrodotoxin. Wakeley et al. (1966) looked for tetrodotoxin in several non-salamandrids—*Necturus maculosus, Amphiuma* sp., *Siren lacertina, Ensatina eschscholtzii, Batrachoseps attenuatus,* and *Aneides lugubris*—but found none. The toxic alkaloids of *Salamandra salamandra* have been studied in great detail (Habermehl 1974), and *Triturus cristatus* has recently been shown to secrete a toxic protein (Jaussi and Kunz 1978).

Skin extracts containing toxins of high molecular weight have been obtained from *Taricha torosa, T. granulosa, Notophthalmus viridescens*, and the plethodontids *Pseudotriton ruber* and *P. montanus* (Brandon and Huheey 1981; Huheey and Brandon 1977). They may also be present in some other plethodontids as well (Brandon and Huheey 1981; Phisalix 1922; Table 1), but skin extracts of most species of salamander remain to be examined. Several other species of plethodontids are demonstrably of reduced palatability to natural and experimental predators (Brodie 1977; Brodie and Howard 1973; Brodie et al. 1979; Dodd and Brodie 1976; Dodd et al. 1974; Huheey 1960). Some species appear to be noxious but not toxic, but to this time skin extracts of all species tested except one of *Desmognathus* and two of *Plethodon* have produced toxic symptoms in bioassay animals, and only some species of *Desmognathus* and some populations of *Plethodon jordani* seem completely palatable to predators.

The objective of this report is to describe the effects of crude skin extracts of *Necturus lewisi* on bioassay mice within the context of similar tests of extracts from a variety of other species.

MATERIALS AND METHODS

Each of two live specimens of *Necturus lewisi* was measured (snout-vent length, mm), weighed (nearest mg), then killed by decapitation and pithed. All skin was dissected from the trunk between hind and front limbs, weighed to the nearest mg, and measured to the nearest mm. The skin was immediately minced and frozen in liquid nitrogen and reduced

Table 1. Bioassay results of white mice injected i.p. with crude skin extracts from two specimens of *Necturus lewisi*.

Dosage (mg/kg)	Result
Animal 1 (98 mm svl, 12.7 g)	
300	moderately strong symptoms
900	strong symptoms
2655	very strong symptoms
7765	very strong symptoms; dead in 2 hr. 20 min.
Animal 2 (123 mm svl, 24.2 g)	
996	very weak symptoms
4671	moderately strong symptoms
9342	strong symptoms
12456	strong symptoms

to a granular powder with a mortar and pestle. The ground sample was slurried in 4-5 ml distilled water for 5 minutes to extract the toxins, then centrifuged for 2 minutes at 3600 rpm, and the supernatant decanted into a sterile centrifuge tube. The residue was rinsed twice with 1-ml portions of distilled water and the supernatant decanted, after centrifugation, into the sterile centrifuge tube. The exact volume was recorded for calculation of dose levels. The sample was kept chilled in ice water at all times except when centrifuged. For all quantitative studies the extract was either tested immediately, or frozen in an ultra-refrigerator (-70 °C) and lyophilized in a Virtis freezedrier for storage. Lyophilized samples from other salamanders were kept at -15 °C in the freezing compartment of a refrigerator and showed no detectable deterioration over time.

Crude extracts were injected i.p. into bioassay mice (Cox Standard Outbred) weighing 18-25 g, in mg of sample per kg bodyweight dosages. Mice were also injected with distilled water, Ringer's solution, and skin extracts of *Desmognathus monticola* as controls; none of these caused symptons. Crude extracts were not toxic enough to yield LD_{50} data but were bioassayed in mice as completely as possible until the sample was expended. Detailed notes were kept of symptoms in bioassay mice to all dosages of crude skin extract.

RESULTS

Following injection of a lethal i.p. dosage of skin extract into a mouse, symptoms appeared within a few minutes and consisted of hind leg stretches (repeated hyperextension of legs and lower back), abdominal compression, occasional hind leg kicks, and wobbly gaint, followed by extreme irritability, then quiescence. The irritability was elicited by contact or impending contact with other mice, a blunt instrument,

sounds, and vibrations. The mice showed a characteristic and distinctive behavior of attempting to escape the stimulus. Leg stretches, abdominal compression, leg kicks, wobbly gait, quiescence, and irritability occurred at all dosages, but most mice seemed gradually to overcome the effects. Relatively larger dosages caused an increase in symptoms and one resulted in death after 2 1/2 hours.

The bioassay data are summarized in Table 2. Although all dosages caused symptoms within the range of 300-12,456 mg/kg, only one mouse died, at a dosage of 7,756 mg/kg.

Table 2. Bioassay results of white mice injected i.p. with crude skin extracts of various salamanders. Dosage expressed as mg salamander skin injected per kg mouse weight.

Species	Dosage (mg/kg)	Result	N
Taricha torosa	20, 27	LD_{50}	2
T. granulosa	48, 75	LD_{50}	2
Notophthalmus viridescens[1] (efts, NC)	143 ± 66	$\overline{X}$ LD_{50}	many
Pseudotriton ruber[2]	235 ± 110	$\overline{X}$ LD_{50}	11
P. montanus[2]	532, 770, 2200,	strong symptoms	4
	3800 and above	lethal	1
Gyrinophilus porphyriticus[2]	up to 14,550	strong symptoms	4
Eurycea longicauda	below 600	strong symptoms	1
	987 to 2673	3/9 lethal	3
E. lucifuga[2]	1,030	strong symptoms	1
	5,080	lethal	1
Desmognathus monticola[2]	up to 5,100	no symptoms	1
Desmognathus brimleyorum	2,824 to 11,296	strong symptoms	2
Plethodon jordani[2]	up to 5,210	no symptoms	1
P. glutinosus[2]	up to 4,000	no symptoms	1
Ambystoma opacum	up to 3,860	symptoms	1
A. texanum	998 to 8,256	strong symptoms	1
controls			
distilled water	up to 4 cc	no symptoms	many

[1] Brandon et al. 1979b
[2] Brandon and Huheey 1981

Symptoms caused by i.p. injections of crude *N. lewisi* skin extract were quite similar to those caused by extracts of *Pseudotriton,* those of several other species of hemidactyline plethodontids, and two species of *Ambystoma*, and the high-molecular weight fractions of newt skin extracts (Brandon and Huheey 1981; Huheey and Brandon 1977; Table 2). We realize these may be generalized symptons of distress, but they are distinct from those caused by tetrodotoxin toxinosis, and they may be characteristic of protein toxins.

In the context of symptomology, the skin of *N. lewisi* is moderately active, in lethality moderately inactive. Without knowledge of the effects of *N. lewisi* skin secretion on natural predators, it is not possible to interpret its effectiveness in predator avoidance. Both Neill (1963) and Shoop and Gunning (1967) found that *Necturus* activity away from cover may be limited by predatory fishes, suggesting that, at least against these predators, skin secretions are not noxious. Experiments of palatability to a variety of potential predators are clearly called for. Antipredator mechanisms have been studied mainly in terrestrial species in which the action of noxious and toxic skin secretions has been reinforced by defensive postures and, often, aposematic coloration, vocalization, and biting (Brodie 1977, 1978). Antipredator mechanisms of permanently aquatic species have not been examined in detail, although some are known to produce noxious and toxic skin secretions (e.g., *Cryptobranchus alleganiensis*, Brodie 1971; Nickerson and Mays 1973; and *Andrias japonicus*, pers. observ.).

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